(FILE 'HOME' ENTERED AT 10:57:15 ON 22 JAN 2003)

FILE 'REGISTRY' ENTERED AT 10:57:26 ON 22 JAN 2003
L1 1 S 75621-03-3

FILE 'CAPLUS' ENTERED AT 10:59:48 ON 22 JAN 2003
L2 823 S L1
L3 1 S L2 AND ISOLATE AND RNA

FILE 'REGISTRY' ENTERED AT 11:00:28 ON 22 JAN 2003

FILE 'CAPLUS' ENTERED AT 11:01:57 ON 22 JAN 2003
L4 1 S L1 AND ISOLATE AND DNA AND DETERGENT
L5 529 S L1 AND DETERGENT
L6 31 S L5 AND (DNA OR RNA OR NUCLEIC ACID)

L6 ANSWER 10 OF 31 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:240107 CAPLUS
DOCUMENT NUMBER: 134:247939
TITLE: Methods for reducing inhibitors in biological samples for **nucleic acid** hybridization and amplification
INVENTOR(S): Little, Michael C.; Llorin, Oscar J.; Collis, Matthew P.
PATENT ASSIGNEE(S): Becton, Dickinson and Company, USA
SOURCE: U.S., 14 pp., Cont.-in-part of U.S. 5,763,185.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6210881	B1	20010403	US 1998-10219	19980121
US 5763185	A	19980609	US 1996-774476	19961230
EP 937780	A1	19990825	EP 1998-124794	19981229
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 11266899	A2	19991005	JP 1999-11259	19990120

PRIORITY APPLN. INFO.: US 1996-774476 A2 19961230
US 1998-10219 A 19980121

AB The invention relates to methods for reducing the amt. of substances inhibitory to **nucleic acid** hybridization in samples for PCR amplification. The method is practiced prior to release of target **nucleic acid** from cells of interest and involves contacting the sample with an agent which solubilizes the inhibitory substances and does not effectuate release of **nucleic acids** from cells in the sample, and then sepn. the cells from the agent. The agents used in the invention include chaotropes such as quinidine thiocyanate, sodium perchlorate and sodium thiocyanate. Also, the sepn. of cells from the agent is generally accomplished by a wash and centrifugation step with a soln. in which the agent sol.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

Applicant

L6 ANSWER 14 OF 31 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:744594 CAPLUS
DOCUMENT NUMBER: 132:89908
TITLE: Modulating restriction endonuclease activities and specificities using neutral **detergents**
AUTHOR(S): Conlan, Lori H.; Jose, Thomas J.; Thornton, Kevin C.; Dupureur, Cynthia M.
CORPORATE SOURCE: Texas A&M University, College Station, TX, USA
SOURCE: BioTechniques (1999), 27(5), 955-958, 960
CODEN: BTNQDO; ISSN: 0736-6205
PUBLISHER: Eaton Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB It is well known that type II restriction enzyme activities and specificities can be modulated by altering soln. conditions. The addn. of co-solvents such as DMSO (DMSO), alcs. and polyols can promote star activity, which is the cleavage of non-cognate sequences. While neutral **detergents** are often used to control protein aggregation, little is known about the effect of neutral **detergents** on restriction enzyme activities and specificities. We report here that BamHI, BglI, BglII, EcoRI, EcoRV, HindIII, MluI, PvuII, SalI and XhoI restriction endonucleases are remarkably tolerant of high concns. of neutral **detergents** Triton X-100, CHAPS and octyl glucoside. In most cases, .lambda. **DNA** cleavage rates were comparable to those obsd. in the absence of **detergent**. Indeed, the specific activities of SalI and XhoI were appreciably increased in the presence of Triton X-100. For all enzymes active in the presence of **detergents**, sequence specificity toward .lambda. **DNA** was not compromised. Assays of star cleavage of pUC18 by EcoRI, PvuII and BamHI endonucleases in equimolar concns. of Triton X-100 and sucrose revealed reduced star activity in the **detergent** relative to the sucrose cosolvent. Interestingly, under star activity-promoting conditions, PvuII endonuclease displayed greater fidelity in Triton X-100 than in conventional buffer. Taken altogether, these results suggest that in some cases, neutral **detergents** can be used to manipulate restriction endonuclease reaction rates and specificities.
REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS

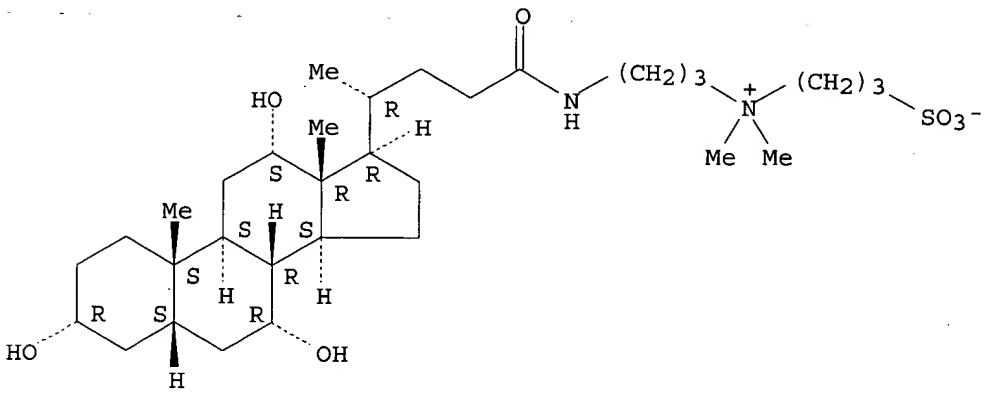
L6 ANSWER 28 OF 31 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:599094 CAPLUS
DOCUMENT NUMBER: 119:199094
TITLE: Enhanced gel mobility shift assay for **DNA** -binding factors
AUTHOR(S): Hassanain, Hamdy H.; Dai, Wei; Gupta, Sohan L.
CORPORATE SOURCE: Hipple Cancer Res. Cent., Dayton, OH, 45439, USA
SOURCE: Analytical Biochemistry (1993), 213(1), 162-7
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Gel mobility shift assays are commonly used to study **DNA**-binding factors involved in the regulation of constitutive, tissue-specific, and inducible genes. The addn. of 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate (Chaps, a zwitterionic **detergent**) at relatively high concn. (.gtoreq.2.5%) to **DNA**-binding reactions for 4 different factors (AP1, SP1, GATA-1, and interferon .alpha.-regulated factor ISGF3) assayed in cell exts. greatly enhanced the signal for **DNA** -protein complexes (up to .apprx.20-fold). The amplified signal for **DNA**-protein complexes so obtained was (at least in part) due to increased binding efficiency, as revealed by greatly reduced amts. of the free probes in the gels. The binding specificity, however, was not compromised. The fact that **DNA**-protein complex formation with 4 different factors was stimulated by Chaps suggests that the enhancing effect of Chaps may be more general and not limited to certain types of **DNA**-binding factors. The results provide the basis for a highly sensitive assay for **DNA**-binding factors, which may be useful in several types of studies on such factors. Among other **detergents** tested, Chapso (another zwitterionic **detergent**), NO-40, and octylglucopyranoside (nonionic **detergents**) also enhanced the complex formation as tested for AP1 binding, whereas sodium cholate and deoxycholate showed strong inhibition.

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS
RN **75621-03-3** REGISTRY
CN 1-Propanaminium, N,N-dimethyl-N-(3-sulfopropyl)-3-

[[(3.alpha.,5.beta.,7.alpha.,12.alpha.)-3,7,12-trihydroxy-24-oxocholan-24-yl]amino]-, inner salt (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Cholane, 1-propanaminium deriv.
OTHER NAMES:
CN 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulphonate
CN 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CN CHAPS
FS STEREOSEARCH
MF C32 H58 N2 O7 S
CI COM
LC STN Files: AGRICOLA, ANABSTR, AQUIRE, BEILSTEIN*, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CANCERLIT, CAPLUS, CHEMCATS, CIN, CSCHEM, DDFU, DRUGU, EMBASE, MEDLINE, MRCK*, MSDS-OHS, NIOSHTIC, PIRA, PROMT, SPECINFO, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.



822 REFERENCES IN FILE CA (1962 TO DATE)
3 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
823 REFERENCES IN FILE CAPLUS (1962 TO DATE)

	Hits	Search Text	DBs	Time Stamp
1	16876	purif$7 with poly(A) with RNA	USPAT	2003/01/17 15:00
2	25483	purif$7 with poly(A) with RNA	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:01
3	4514	purif$7 near5 poly(A) adj RNA	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:01
4	2034268	poly(dT) or poly(U)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
5	4422	l3 and l4	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
6	4050	l3 with l4	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
7	0	l3 with l4l6 with hybrize	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
8	0	l3 with l4l6 with hybridize	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
9	18	l3 with l6 with hybridize	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:03

	Hits	Search Text	DBs	Time Stamp
1	16876	purif$7 with poly(A) with RNA	USPAT	2003/01/17 15:00
2	25483	purif$7 with poly(A) with RNA	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:01
3	4514	purif$7 near5 poly(A) adj RNA	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:01
4	2034268	poly(dT) or poly(U)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
5	4422	(purif$7 near5 poly(A) adj RNA) and (poly(dT) or poly(U))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:06
6	0	(purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U)) with hybrize	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
7	0	(purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U)) with hybridize	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:02
8	18	(purif$7 near5 poly(A) adj RNA) with ((purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U))) with hybridize	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:03
9	4422	(purif$7 near5 poly(A) adj RNA) and (poly(dT) or poly(U))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:06
10	48	(purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U))and (tetramethylammonium adj1 chloride or tetraethylammonium adj chloride or TEAC or TMAC)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/17 15:10

	Hits	S arch T xt	DBs
1	16876	purif$7 with poly(A) with RNA	USPAT
2	25483	purif$7 with poly(A) with RNA	USPAT; US-PGPUB; EPO; JPO; DERWENT
3	4514	purif$7 near5 poly(A) adj RNA	USPAT; US-PGPUB; EPO; JPO; DERWENT
4	2034268	poly(dT) or poly(U)	USPAT; US-PGPUB; EPO; JPO; DERWENT
5	4422	(purif$7 near5 poly(A) adj RNA) and (poly(dT) or poly(U))	USPAT; US-PGPUB; EPO; JPO; DERWENT
6	0	(purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U)) with hybrize	USPAT; US-PGPUB; EPO; JPO; DERWENT
7	0	(purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U)) with hybridize	USPAT; US-PGPUB; EPO; JPO; DERWENT
8	18	(purif$7 near5 poly(A) adj RNA) with ((purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U))) with hybridize	USPAT; US-PGPUB; EPO; JPO; DERWENT
9	4422	(purif$7 near5 poly(A) adj RNA) and (poly(dT) or poly(U))	USPAT; US-PGPUB; EPO; JPO; DERWENT
10	48	(purif$7 near5 poly(A) adj RNA) with (poly(dT) or poly(U))and (tetramethylammonium adj1 chloride or tetraethylammonium adj chloride or TEAC or TMAC)	USPAT; US-PGPUB; EPO; JPO; DERWENT
11	7008	tetramethylammonium adj1 chloride or tetraethylammonium adj chloride or TEAC or TMAC	USPAT; US-PGPUB; EPO; JPO; DERWENT
12	4428	purif$7 near5 poly(A) adj RNA) and (poly(dT) or poly(U)	USPAT; US-PGPUB; EPO; JPO; DERWENT
13	61	(tetramethylammonium adj1 chloride or tetraethylammonium adj chloride or TEAC or TMAC) and (purif$7 near5 poly(A) adj RNA) and (poly(dT) or poly(U))	USPAT; US-PGPUB; EPO; JPO; DERWENT
14	1	("5759777").PN.	USPAT
15	1	("5759777").PN.	USPAT
16	2986	((purify or purification or isoloate) near5 poly(A) adj RNA) and (poly(dT) or poly(U))	USPAT; US-PGPUB; EPO; JPO; DERWENT
17	786	(((purify or purification or isoloate) near5 poly(A) adj RNA) and (poly(dT) or poly(U))) and biotinylat$3	USPAT; US-PGPUB; EPO; JPO; DERWENT

	Time Stamp
1	2003/01/22 10:15
2	2003/01/17 15:01
3	2003/01/17 15:01
4	2003/01/17 15:02
5	2003/01/22 08:28
6	2003/01/17 15:02
7	2003/01/17 15:02
8	2003/01/17 15:03
9	2003/01/22 10:15
10	2003/01/22 08:27
11	2003/01/22 08:28
12	2003/01/22 08:32
13	2003/01/22 08:32
14	2003/07/23 10:03
15	2003/01/22 09:42
16	2003/01/22 10:18
17	2003/01/22 10:22

	Hits	Search T xt	DBs
18	155	((((purify or purification or isoloate) near5 poly(A) adj RNA) and (poly(dT) or poly(U))) and (biotinylat$3 with (poly(a) poly(t) poly(u) oligo(dt)))) and (poly(a) or poly(t) with hybridization)	USPAT; US-PGPUB; EPO; JPO; DERWENT
19	4	(((purify or purification or isoloate) near5 poly(A) adj RNA) and (poly(dT) or poly(U))) and (biotinylat$3 with (poly(a) poly(t) poly(u) oligo(dt)) with hybridization)	USPAT; US-PGPUB; EPO; JPO; DERWENT
20	155	(((purify or purification or isoloate) near5 poly(A) adj RNA) and (poly(dT) or poly(U))) and (biotinylat$3 with (poly(a) poly(t) poly(u) oligo(dt)))	USPAT; US-PGPUB; EPO; JPO; DERWENT
21	137	((((purify or purification or isoloate) near5 poly(A) adj RNA) and (poly(dT) or poly(U))) and (biotinylat$3 with (poly(a) poly(t) poly(u) oligo(dt)))) and (avidin or streptavidin)	USPAT; US-PGPUB; EPO; JPO; DERWENT
22	16	(((purify or purification or isoloate) near5 poly(A) adj RNA) and (poly(dT) or poly(U))) and (biotinylat$3 with (poly(a) poly(t) poly(u) oligo(dt)) with (avidin or streptavidin))	USPAT; US-PGPUB; EPO; JPO; DERWENT

	Time Stamp
18	2003/01/22 10:24
19	2003/01/22 10:24
20	2003/01/22 10:34
21	2003/01/22 10:34
22	2003/01/22 10:37

PGPUB-DOCUMENT-NUMBER: 20010014451
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010014451 A1
TITLE: Nucleic acid detection
PUBLICATION-DATE: August 16, 2001

INVENTOR-INFORMATION:

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APPL-NO: 09/ 757132
DATE FILED: January 9, 2001

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INT-CL: [07], C12Q001/68
US-CL-PUBLISHED: 435/6, 536/23.1
US-CL-CURRENT: 435/6, 536/23.1

ABSTRACT:

This invention discloses methods, compositions and kits for the detection of extremely low levels of nucleic acid, cells and cellular material in biological samples. The nucleic acid detection systems utilize either the pyrophosphorolysis reaction catalyzed by various polymerases or nuclease digestion coupled with pyrophosphorylation catalyzed by phosphoribosylpyrophosphate synthetase to produce either deoxyribonucleoside triphosphates or ribonucleoside triphosphates. dNTPs are transformed to ATP by the action of nucleoside diphosphate kinase. The ATP produced by these reactions may be detected by luciferase or NADH based detection systems. If more sensitive detection is required, schemes for the amplification of NTPs and

dNTPS are provided. A detection system for cells or cellular material in a sample is provided wherein AMP and a high energy phosphate donor added to a sample are converted to ATP by the action of endogenous enzymes, followed by detection of the ATP.

Summary of Invention Paragraph - BSTX:

[0005] A third method of detecting low levels of DNA utilizes biotinylated single-stranded DNA binding protein (SSB), streptavidin, an anti-DNA antibody fused to urease, and biotinylated nitrocellulose as reagents. This assay is commercially available from Molecular Devices and described in Kung et al., Picogram Quantitation of Total DNA Using SNA-Binding Proteins in a Silicon Sensor-Based System, Anal. Biochem. 187: 220-27 (1990). The assay is performed by incubating the streptavidin, biotin-SSB, and the anti-DNA antibody together, allowing a complex to be formed. The complex is then captured on the biotinylated filter, washed, and the amount of captured urease is read. This method is highly sensitive but has several disadvantages. These disadvantages include costly reagents and the need for extensive controls.

Summary of Invention Paragraph - BSTX:

[0007] U.S. Pat. No. 4,735,897 describes a method of detecting polyadenylated messenger RNA (poly(A)-mRNA). Depolymerization of poly (A)-mRNA in the presence of phosphate has been shown to result in the formation of ADP, which can be converted by pyruvate kinase or creatine phosphokinase into ATP. RNA may also be digested by a ribonuclease to AMP, converted to ADP by adenylate kinase, and then converted to ATP by pyruvate kinase.

Summary of Invention Paragraph - BSTX:

[0016] catalyzed by poly(A) polymerase. In a quantitative assay for RNA, the depolymerizing step is repeated essentially to completion or equilibrium to obtain at least two nucleoside triphosphate molecules from a strand of minimally three nucleotides. For detection of DNA, the depolymerizing step need not be repeated if there are sufficient nucleic acid molecules present to generate a signal. The ATP molecules so formed are then detected with either a luciferase detection system or a NADH detection system. The sensitivity of the reaction may be increased by optionally amplifying the ATP molecules.

Summary of Invention Paragraph - BSTX:

[0017] In another embodiment of the present invention, a method is

provided for selectively detecting and/or assaying poly(A) mRNA in a reaction containing pyrophosphate, adenosine 5'-diphosphate, or a combination thereof. In this method, a complementary oligo (dT) probe is hybridized to poly(A) mRNA to form an RNA-DNA hybrid. The oligo (dT) strand of the RNA-DNA hybrid is then depolymerized at the terminal nucleotide by enzymatically cleaving the terminal internucleotide phosphodiester bond and reforming same with a pyrophosphate molecule to form deoxythymidine 5'-triphosphate. According to the following reaction:

Summary of Invention Paragraph - BSTX:

[0036] The present invention provides various kits for nucleic acid detection. First, a kit is provided which contains reagents for the detection of DNA by pyrophosphorolysis. The kit contains a vessel containing a nucleic acid polymerase and a vessel containing a nucleoside disphosphate kinase. The nucleic acid polymerase and nucleoside diphosphate kinase may be provided in the same container. Second, a kit is provided which contains reagents for the detection of nucleic acid by nuclease digestion. The kit contains a vessel containing phosphoribosylpyrophosphate synthetase and a vessel containing a nuclease. Third, a kit is provided which contains reagents for the detection of RNA by pyrophosphorolysis. The kit contains a vessel containing poly(A)-polymerase. Fourth, a kit containing reagents for the detection of DNA by nuclease digestion is provided. This kit contains a vessel containing phosphoribosylpyrophosphate synthetase and a vessel containing nucleoside disphosphate kinase. The phosphoribosylpyrophosphat- e synthetase and nucleoside diphosphate kinase may optionally be provided in the same container.

Summary of Invention Paragraph - BSTX:

[0050] Several polymerases are also known to catalyze the reverse of the polymerization process. This reverse reaction is called pyrophosphorolysis. The pyrophosphorolysis activity of DNA polymerase was demonstrated by Deutscher and Kornberg, Enzymatic Synthesis of Deoxyribonucleic Acids, J. Biol. Chem. 244: 3019-28 (1969). Other nucleic acid polymerases capable of pyrophosphorolysis include DNA polymerase .alpha., DNA polymerase .beta., T4 DNA polymerase, Taq polymerase, Klenow fragment, AMV reverse transcriptase, and MMLV reverse transcriptase. However, not all polymerases are known to possess pyrophosphorolysis activity. For example, poly(A) polymerase has been reported to not catalyze pyrophosphorylation. (See Sippel, Eur. J. Biochem. 37:31-40 (1973).)

Summary of Invention Paragraph - BSTX:

[0061] Applicants have further demonstrated that poly(A) polymerase may catalyze pyrophosphorolysis, even though no such

reaction has been previously reported. In fact, poly(A) polymerase has been widely reported to not catalyze pyrophosphorolysis. See, for example, Sippel, Eur. J. Biochem. 37:31-40 (1973) and Sano and Feix, Eur. J. Biochem. 71:577-83 (1976). Surprisingly, the applicants show that under the proper reaction conditions poly(A) polymerase catalyzes phosphorolysis. Preferably, the manganese chloride present in the previously reported buffers is omitted, the concentration of sodium chloride is decreased, and the pH is lowered from about 8.0 to about 7.5. Most preferably, poly(A) polymerase pyrophosphorolysis reaction buffer contains about 50 mM Tris-Cl pH 7.5, 10 mM MgCl.sub.2, 50 mM NaCl, and 2 mM NaPP.sub.i (sodium pyrophosphate).

Summary of Invention Paragraph - BSTX:

[0063] In a preferred embodiment of the present invention for detecting nucleic acids, nucleic acid polymerase and PP.sub.i are added to a sample containing less than 1 .mu.g nucleic acid, down to less than about 10 pg of nucleic acid. To increase the sensitivity of the DNA detection, the DNA may be fragmented by treatment with a restriction endonuclease or by sonication. Next, the nucleic acid is degraded by pyrophosphorolysis releasing free NTPs or dNTPs. Enzymes useful in the pyrophosphorolysis reaction include AMV reverse transcriptase, MMLV reverse transcriptase, DNA polymerase alpha and beta, Taq polymerase, T4 DNA polymerase, Klenow fragment and poly(A) polymerase. Most preferably, T4 polymerase is utilized for DNA pyrophosphorolysis reactions because of its recognition of 3' and 5' overhangs and blunt ends and high processivity as noted above.

Summary of Invention Paragraph - BSTX:

[0068] After digestion with the nuclease, the NMPs or dNMPs are converted to NTPs or dNTPs respectively. U.S. Pat. No. 4,375,897 describes the detection of RNA by digestion with nucleases followed by conversion to NTP. This method utilizes a two-step scheme in which adenylate kinase converts AMP to ADP, and pyruvate kinase then converts ADP to ATP. This method is essentially limited to the detection of poly(A) mRNA because no mechanism is suggested for conversion of dNTPs to ATP, the preferred substrate for luciferase. Nuclease digestion or phosphorolysis of DNA results in a mixture of dNTPs which do not act as efficient substrates for luciferase.

Summary of Invention Paragraph - BSTX:

[0096] The pyrophosphorolysis reaction and amplification reaction may also be performed in a single pot reaction. In this single pot reaction, the polymerases may be AMV reverse transcriptase, MMLV reverse transcriptase, DNA polymerase alpha or beta, Taq polymerase, T4 DNA polymerase, Klenow fragment or poly(a) polymerase, a first enzyme for converting AMP to ADP may be myokinase (adenylate kinase) or NMPK, and a second enzyme for converting ADP to ATP may be

pyruvate kinase or NDPK. The reaction must be fed AMP, preferably Apyrase treated AMP so that background due to contaminating ADP and ATP is minimized. Preferably 1 .mu.l of 1U/.mu.l Apyrase may be added to 19 .mu.l of 10 mM AMP, followed by incubation at room temperature for 30 minutes and heat inactivation of the Apyrase by incubation at 70.degree. C. for 10 minutes. High energy phosphate donors must also be added to the reaction. When pyruvate kinase is utilized phosphoenolpyruvate is added, while when NDPK is utilized dCTP is added. Preferably, the high energy phosphate donor is added about 15 minutes after a preincubation with the polymerase, although this is not necessary. These reactions may characterized as follows:

Summary of Invention Paragraph - BSTX:

[0098] In another embodiment, the reactions described above may be used to selectively detect poly(A) mRNA according to the following scheme. First oligo(dT) primers are hybridized to the poly(A) tails of the mRNA to form a DNA-RNA hybrid. Next, a pyrophosphorolysis reaction is performed using reverse transcriptase (RT). Reverse transcriptases which may be used in the present invention include Mouse Mammary Leukemia Virus (MMLV) RT, Avian Myeloma Virus (AMV) RT and Rous Sarcoma Virus (RSV) RT. An advantage of this detection system is that these RTs catalyze pyrophosphorolysis of double stranded nucleic acid and double stranded RNA-DNA hybrids, but not single stranded nucleic acids. Thus, the amount of poly(A) RNA in a total cellular RNA sample be determined. The pyrophosphorolysis reaction produces dTTP according to the following reaction:

Summary of Invention Paragraph - BSTX:

[0099] wherein TT.sub.n is oligo(dT) and PP.sub.i is pyrophosphate.

Summary of Invention Paragraph - BSTX:

[0107] In another aspect of the present invention, a nucleic acid detection test kit is provided for performing the pyrophosphorolysis nucleic acid detection method. The nucleic acid detection test kit comprises the essential reagents required for the method of the nucleic acid detection invention. For nucleic acid detection by pyrophosphorolysis, the kit includes a vessel containing an enzyme capable of catalyzing pyrophosphorolysis such as Taq polymerase, T4 polymerase, AMV reverse transcriptase, MMLV reverse transcriptase, or poly(A) polymerase. The concentration of polymerase is 0.1 to 100 units/.mu.l, preferably about 5 units/.mu.l. Kits for use in DNA detection also include a vessel containing nucleoside diphosphokinase and a vessel containing ADP. Preferably, these reagents are free of contaminating ATP and adenylate kinase. The NDPK is provided in concentration of about 0.1 to 100 units/.mu.l, preferably about 1.0 units/.mu.l. The contaminants may be removed by dialysis or Apyrase

treatment. Optionally, the kit may contain vessels with reagents for amplification of dNTPs or NTP to ATP. Amplification reagents include pyruvate kinase, adenylate kinase, NMPK, NDPK, AMP as the amplification substrate, and dCTP or AMP-CPP as high-energy phosphate donors. The kit may be packaged in a single enclosure including instructions for performing the assay methods. The reagents are provided in containers and are of a strength suitable for direct use or use after dilution. A standard set may also be provided to allow quantitation of results. Test buffers for optimal enzyme activity may be included. Most preferably, the NDPK and nucleic acid polymerase are provided in the same reaction mix so that a single pot reaction may be performed consistently.

Detail Description Paragraph - DETX:

[0167] At time equals zero minutes of digestion, 10 .mu.l of each of these was removed and added to 490 .mu.l of 50 mM Tris Cl pH 8.0. Immediately, 1 .mu.l of S1 nuclease was added to the remaining reaction mixtures 1 and 3 but not 2, and the mixtures were allowed to incubate at room temperature. Additional 10 .mu.l samples of the reactions were removed after 20, 50 and 140 min of reaction and diluted into 490 .mu.l of 50 mM Tris Cl pH 8.0. The data is presented in Table 25. The absorbance of the solution in Reaction #1 increased, again indicating that the polymer in this reaction was digested over time. A second set of reactions was produced as described above. The only difference with these reactions was that 50 units of Sigma Poly (dA) (Sigma P-0887, Lot #67H0226) was dissolved in 1.5 ml of TE buffer and used in the reactions. After the 140 minutes of digestion, these reactions were used as described in Example 16.

Detail Description Paragraph - DETX:

Detection of Poly (dA) using nucleases and PRPP Synthetase

Detail Description Paragraph - DETX:

[0169] Table 26 presents the components of the PRPP Synthetase reaction. The concentrations of the components were: PRPP, 2.6.times.10.sup.-4 M in 10 mM Tris-Cl pH 7.5; PRPP Synthetase, 6.times.10.sup.31 4 Units of Sigma P0287 per 2 .mu.l in PRPP Synthetase Buffer. For composition of Buffer, refer to PRPP Synthetase Buffer in Example 13. The nucleoside digests containing S1 were diluted in deionized water to yield the amount of polymer listed in the Table in 8 .mu.l of solution and added to the appropriate reactions. The digest containing no polymer was diluted identically to those with polymer. Eight microliters of this solution contained all the components in the samples containing 720ng of polymer except the Poly(dA). All the reactions were incubated 32 min in a 37.degree. C. water bath. At this point all the reactions were heated at 95.degree. C. for 5 min to inactivate the PRPP Synthetase and cooled

in an ice bath for 5 min.

Detail Description Paragraph - DETX:

[0182] This example demonstrates the detection of DNA by digestion of the polymer to nucleoside monophosphates using nucleases, transformation of the nucleoside monophosphates to nucleoside triphosphates using PRPP Synthetase and PRPP along with transformation of ADP to ATP using the nucleoside triphosphates generated by the action of PRPP Synthetase, and detection of the ATP using Luciferase. A sample of deoxynucleoside (Poly (dA)) was prepared as described in example 17. Different amounts of deoxynucleoside were used in the reactions as presented in Table 30.

Detail Description Paragraph - DETX:

Detection of PolyA RNA using Poly A Polymerase

Detail Description Paragraph - DETX:

[0196] This example demonstrates the detection Poly A mRNA by the pyrophosphorylation of the poly A segment. The reactions were assembled as demonstrated in Table 36. The compositions of the reaction materials was: 10X Buffer-0.5M Tris-HCl, pH 7.5, 0.1 M MgCl.sub.2, 0.5 M NaCl; Globin mRNA GibcoBRL cat#18103-028 (dissolved in H.sub.2O); NaPP.sub.I, 20 mM sodium pyrophosphate (Promega C113A, in deionized water); Poly A Polymerase, (Sigma P4058, 1U/.mu.l). These reactions were incubated at 37.degree. C. for 30 min, then 2 .mu.l of the reaction was added to 100 .mu.l of L/L Reagent and the light output of the reaction immediately measured using a Turner TD-20e Luminometer. The data is presented in Table 37. These data demonstrate that Poly A Polymerase is capable of pyrophosphorylating the RNA and that the resulting nucleoside triphosphates can be detected using luciferase, even if only very low levels of RNA are present.

Detail Description Paragraph - DETX:

Detection of Poly A RNA using Reverse Transcriptase and NDPK

Detail Description Paragraph - DETX:

[0199] The reactions were assembled as presented in Table 38. The reaction components were: Buffer, 5X MMLV-RT Buffer (Promega Part #M531A, Lot #7090101); mRNA, Globin mRNA (GibcoBRL cat# 18103-028 dissolved in H.sub.2O); Poly (dT), 0.2 .mu.M oligo dT(50), NaPPi, 20mM Sodium Pyrophosphate, (Promega C113A in deionized water); ADP, 10 mM ADP (Sigma A-5285 Lot #56H7815); NDKP, nucleoside diphosphate kinase, 1U/.mu.l, (Sigma N-0379 Lot #127F81802); MMLV-RT, (Promega

Part #M531A, Lot #7090101) 200U/.mu.l; and 200U/.mu.l Superscript II GibcoBRL cat# 18064-014).

Detail Description Table CWU - DETL:

27TABLE 26 Reac- PRPP tion Digest Buffer PRPP Synthetase poly(dA) S1 1 720 ng 80 .mu.l 2 .mu.l 2 .mu.l -- -- 2 72 ng 80 .mu.l 2 .mu.l 2 .mu.l -- -- 3 7.2 ng 80 .mu.l 2 .mu.l 2 .mu.l -- -- 4 0.72 ng 80 .mu.l 2 .mu.l 2 .mu.l -- -- 5 -- 80 .mu.l 2 .mu.l 2 .mu.l 720 ng -- 6 -- 80 .mu.l 2 .mu.l 2 .mu.l -- (720 ng

Detail Description Table CWU - DETL:

28TABLE 27 Light Units Reaction DNA no NDPK w/NDPK 1 180 ng 43 711 2 18 ng 15 227 3 1.8 ng 13 77 4 0.18 ng 11 37 5 no S1 13 161 6 no poly 11 28

Detail Description Table CWU - DETL:

29TABLE 28 (Reaction Components**) Reaction DNA LAR-CoA AMP PEP AK PK Tris PRPP Butter 1 poly(dA) 180 ng, 20 .mu.l* + + + + + -- -- 2 poly(dA) 18 ng, 20 .mu.l* + + + + + -- -- 3 poly(dA) 1.8 ng, 20 .mu.l* + + + + + -- -- 4 poly(dA) + + + + + -- -- 5 poly(dA) no S1 + + + + + -- -- 6 Si nuclease, no + + + + + -- -- 7 ATP 14 .mu.l 2 mM + + + + + -- 6 .mu.l 8 dATP 14 .mu.l 2 nM + + + + + -- 6 .mu.l 9 dATP 14 .mu.l 200 nM + + + + + -- 6 .mu.l 10 dATP 14 .mu.l 20 nM + + + + + -- 6 .mu.l 11 none + + + + + 14 .mu.l 6 .mu.l *These reactions used 20 .mu.l of the heat-inactivated PRPP Synthetase reactions from the first part of this example. **The components were: ATP (Sigma A9187) in 10 mM Tris pH 7.5, dATP (Sigma D6500) in 10 mM Tris pH 7.5, AMP 7 .mu.l of 2 .times. 10.sup.-4M in 10 mM Tris pH 7.5, LAR-CoA (LAR without CoA) 700 .mu.l per reaction tube, PEP (phosphoenol pyruvate-ammonium salt) (synthesized) 7 .mu.l of 100 mM, AK (adenylate kinase/myokinase) (Sigma M5520) 14 .mu.l of 0.75 units/.mu.l in Buffer A, PK (pyruvate kinase) (Sigma P7286, dialyzed 48 hours) 17.5 .mu.l of 0.13 units/.mu.l, Tris 10 mM pH 7.5, PRPP Synthetase Buf #fer-see example 13.

Detail Description Table CWU - DETL:

37TABLE 36 Reaction 10X Globin NaPPi Poly A Water 1 2 .mu.l 1 .mu.l of 50 ng/.mu.l 1 .mu.l 1 .mu.l 15 .mu.l 2 2 .mu.l 1 .mu.l of 10 ng/.mu.l 1 .mu.l 1 .mu.l 15 .mu.l 3 2 .mu.l 1 .mu.l of 2 ng/.mu.l 1 .mu.l 1 .mu.l 15 .mu.l 4 2 .mu.l 1 .mu.l of 400 pg/.mu.l 1 .mu.l 1 .mu.l 15 .mu.l 5 2 .mu.l 1 .mu.l of 80 pg/.mu.l 1 .mu.l 1 .mu.l 15 .mu.l 6 2 .mu.l 1 .mu.l of 16 pg/.mu.l 1 .mu.l 1 .mu.l 15 .mu.l 7 2 .mu.l -- 1 .mu.l 1 .mu.l 15 .mu.l

Detail Description Table CWU - DETL:

39TABLE 38 Rx Suffer mRNA Poly (dT) NaPPi APP NDPK MMLV-RT Superscript water 1 4 .mu.l 1 .mu.l of 50 ng/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l 1 .mu.l -- 9 .mu.l 2 4 .mu.l 1 .mu.l of 10 ng/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l 1 .mu.l -- 9 .mu.l 3 4 .mu.l 1 .mu.l of 2 ng/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l 1 .mu.l -- 9 .mu.l 4 4 .mu.l 1 .mu.l of 400 pg/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l 1 .mu.l -- 9 .mu.l 5 4 .mu.l 1 .mu.l of 80 pg/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l 1 .mu.l -- 9 .mu.l 6 4 .mu.l -- 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l 1 .mu.l -- 9 .mu.l 7 4 .mu.l 1 .mu.l of 50 ng/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l -- 1 .mu.l 9 .mu.l 8 4 .mu.l 1 .mu.l of 10 ng/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l -- 1 .mu.l 9 .mu.l 9 4 .mu.l 1 .mu.l of 2 ng/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l -- 1 .mu.l 9 .mu.l 10 4 .mu.l 1 .mu.l of 400 pg/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l -- 1 .mu.l 9 .mu.l 11 4 .mu.l 1 .mu.l of 80 pg/.mu.l 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l -- 1 .mu.l 9 .mu.l 12 4 .mu.l -- 1 .mu.l 1 .mu.l 2 .mu.l 1 .mu.l -- 1 .mu.l 9 .mu.l

Claims Text - CLTX:

9. A method of detecting polyadenylated mRNA in a reaction containing pyrophosphate, the method comprising: depolymerizing the polyadenylated mRNA at a terminal nucleotide by enzymatically cleaving the terminal internucleotide phosphodiester bond and reforming same with a pyrophosphate molecule to form a free adenosine triphosphate molecule according to the reaction catalyzed by poly(A) polymerase: NA.sub.n+PP.sub.i.fwdarw.NA.sub.n-1+ATP; and detecting the adenosine 5'-triphosphate formed thereby.

Claims Text - CLTX:

14. A method of selectively detecting poly(A)-mRNA in a reaction containing pyrophosphate, adenosine 5'-diphosphate, or a combination thereof, the method comprising: hybridizing a complimentary oligo(dT) probe to poly(A)-mRNA to form a RNA-DNA hybrid, depolymerizing the oligo(dT) strand of the RNA-DNA hybrid at the terminal nucleotide by enzymatically cleaving the terminal internucleotide phosphodiester bond and reforming same with a pyrophosphate molecule to form a deoxythymidine 5'-triphosphate molecule according to the following general reaction catalyzed by a reverse transcriptase: dTT.sub.n+PP.sub.i.fwdarw.dTT.sub.n- -1+dTTP; enzymatically transferring terminal 5' phosphate groups from the deoxythymidine 5'-triphosphate molecules to adenosine 5'-diphosphate molecules to form adenosine 5'-triphosphate molecules according to the following general reaction: dTTP*+ADP=dTDP+ATP*, wherein P* is the terminal 5' phosphate so transferred; and detecting the adenosine 5'-triphosphate formed thereby.

Claims Text - CLTX:

46. A kit containing reagents for the detection of RNA by

pyrophosphorolysis comprising: a vessel containing poly(A)-polymerase.

Claims Text - CLTX:

56. A method of assaying polyadenylated mRNA in a reaction containing pyrophosphate, the method comprising: depolymerizing the polyadenylated mRNA at a terminal nucleotide by enzymatically cleaving the terminal internucleotide phosphodiester bond and reforming same with a pyrophosphate molecule to form a free adenosine triphosphate molecule according to the reaction catalyzed by poly(A) polymerase: NA.sub.n+PP.sub.i.fwdarw.NA.sub.n-1+ATP; repeating the depolymerizing step to obtain at least 2 nucleoside triphosphate molecules; and detecting the adenosine 5'-triphosphate formed thereby.

Claims Text - CLTX:

61. A method of selectively assaying poly(A)-mRNA in a reaction containing pyrophosphate, adenosine 5'-diphosphate, or a combination thereof, the method comprising: hybridizing a complimentary oligo(dT) probe to poly(A)-mRNA to form a RNA-DNA hybrid, depolymerizing the oligo(dT) strand of the RNA-DNA hybrid at the terminal nucleotide by enzymatically cleaving the terminal internucleotide phosphodiester bond and reforming same with a pyrophosphate molecule to form a deoxythymidine 5'-triphosphate molecule according to the following general reaction catalyzed by a reverse transcriptase: dTT.sub.n*+PP.sub.i.fwdarw.dTT.sub.- n-1+dTTP; repeating the depolymerizing step to obtain at least two nucleoside triphosphate molecules; enzymatically transferring terminal 5' phosphate groups from the deoxythymidine 5'-triphosphate molecules to adenosine 5'-diphosphate molecules to form adenosine 5'-triphosphate molecules according to the following general reaction: dTTP*+ADP=dTDP+ATP*, wherein P* is the terminal 5' phosphate so transferred; and detecting the adenosine 5'-triphosphate formed thereby.

SIGMA

Sigma Product Information Sheet

TRITON X-100™

Sigma product number X-100

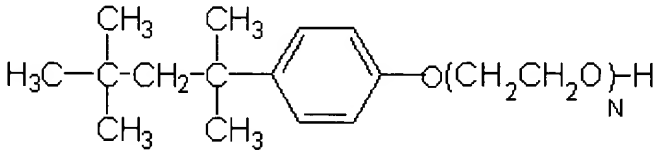
CAS NUMBER:
9002-93-1

NAMES:
X-100; Triton X-100; octylphenol ethylene oxide condensate; Octoxynol-9
[Triton X-100 was a registered trademark formerly owned by Rohm and Haas Co., but now owned by Union Carbide.]

GENERAL INFORMATION:
X-100 is a nonionic detergent, 100% active ingredient, which is often used in biochemical applications to solubilize proteins. Triton X-100 has no antimicrobial properties.(1) It is considered a comparatively mild detergent, non-denaturing, and is reported in numerous references as a routinely added reagent. It does absorb in the ultraviolet region of the spectrum, however, so can interfere with protein quantitation. A number of polymeric resins have been used to remove X-100 from solution, including Amberlite hydrophobic XAD resins(7) and Rezorian A161 cartridges.(2)

The "X" series of Triton detergents are produced from octylphenol polymerized with ethylene oxide. The number ("-100") relates only indirectly to the number of ethylene oxide units in the structure. X-100 has an "average of 9.5" ethylene oxide units per molecule, with an average molecular weight of 625. (1,2) In addition, lower and higher mole adducts will be present in lesser amounts, varying slightly within supplier's standard manufacturing conditions. (A by-product formed during the reaction is polyethylene glycol, a homopolymer of ethylene oxide. Acid is also added to the product to neutralize the product after the base catalyzed reaction is completed.) No antioxidants are added by Sigma or the manufacturer, but commercial preparations of Triton X-100 have been found to contain peroxides up to 0.22% hydrogen peroxide (H2O2) equivalents.(8) These impurities may interfere with biological reactions. Sigma offers X-100-PC and X-100R-PC as biological grade alternatives.

STRUCTURE:



N = approx. 9.5

Triton X-100 has a structure very similar to those of Igepal CA-630 [Sigma I3021] and of Nonidet P-40 (no longer commercially available), and the names are sometimes reported as synonyms.(3) However, Triton X-100 is slightly more hydrophilic than Igepal CA-630; these two detergents are NOT considered to be functionally interchangeable in most applications.

PHYSICAL PROPERTIES:

(per supplier unless indicated otherwise)

Triton X-100 is a very stable material, assumed to be stable for years if stored sealed. It is a clear to slightly hazy, colorless to light yellow liquid (color by APHA = 100).

Specific gravity: 1.065 at 25°C (Approx. 1.07 g/mL)
Approximate molecular weight = 625, giving effective molarity = 1.7 M for the neat liquid.
UV absorption: lambda max = 275 nm and 283 nm in methanol (4)
Sigma average data: for lambda = 277 nm, E1% = 23.9; for lambda = 283 nm, E1% = 19.4.

Typical values:
Viscosity (Brookfield): 240 cps at 25°C (Sigma values are usually somewhat higher.)(2)
Pour point: 7°C
pH (5% aqueous solution): 6.0 to 8.0
Cloud point (1% aqueous solution): 63-69°C
Calculated HLB value: 13.5
Critical micelle concentration (CMC): 0.22 to 0.24 mM (2,5,6)

SOLUBILITY / STABILITY:
Triton X-100 is soluble in all proportions at 25°C in water, benzene, toluene, xylene, trichloroethylene, ethylene glycol, ethyl ether, ethanol, isopropanol, and ethylene dichloride. Sigma assays dissolves 1 mL X-100 in 10 mL water to give a clear to slightly hazy solution, from clear to slightly yellow in appearance.

Solutions are stable to autoclaving. At certain concentrations the solutions may be cloudy but dispersible above the cloud point; they should clear with stirring upon cooling. However, any ethylene oxide polymer can form trace peroxides on exposure to oxygen. These impurities may interfere with biological reactions. Sigma offers X-100-PC and X-100R-PC as biological grade alternatives.

SPECIFIC USAGE NOTES:
For lysing cells, typically about 0.1% X-100 solution in water will be sufficient, and even up to 0.5% concentrations will usually not harm most enzymes being isolated.(2) Many enzymes remain active in the presence of X-100; for example, a commonly used protease, Proteinase K, remains active in 1% (w/w) solutions of X-100.(9)

APPLICATIONS AND REFERENCES:
Solubilization of membranes by detergents:
BIOCHIM. BIOPHYS. ACTA, 553, 40 (1979).
BIOCHIM. BIOPHYS. ACTA, 415, 29 (1975).

Hydrogenation of Triton X-100:
ANAL. BIOCHEM., 141, 262 (1984).

Detergent effects on enzyme activity:
BIOCHIM. BIOPHYS. ACTA, 733, 210 (1983).

Solubilization of the human erythrocyte membrane:
BIOCHEM. J., 164, 465 (1977).

Use in scintillation counting:

INT. J. APPL. RADIAT. ISOTOPES, 20, 499 (1969).

CITED REFERENCES:

1. Supplier data (Union Carbide Company).
2. Sigma data.
3. MERCK INDEX, 11th ed. #6681 (1989).
4. ANAL. CHEM., 35, 1943 (1963).
5. BIOCHIM. BIOPHYS. ACTA, 415, 29 & 39 (1975).
6. ANAL. BIOCHEM., 141, 262 (1984).
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8. ANAL. BIOCHEM., 109, 55-62 (1980).
9. ENZYMES OF MOLECULAR BIOLOGY [Sigma E1144], M. M. Burrell, Ed., Humana Press, (Totowa, NJ, 1993), p. 307.

CKV/012196/T6878.gen

Apoptosis
Cancer Research
Cell Biology
Molecular Biology
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Signal Transduction
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Amino Acid
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CHAPS [C1019]

Description: Zwitterionic detergent that combines the features of bile salts and N-alkyl sulfobetaines. Can be easily removed from gels or protein solutions by dialysis across a membrane.
Chemical Formula: C32H58N2O7S
Chemical Name: : 3-[3-(Cholamidopropyl)dimethylammonio]-1-proanesulfonate
Molecular Weight: M.W. 614.9
Solubility: Soluble in Water.
CAS Number: CAS [75621-03-3]
Active Product: Yes
Appearance: White Solid
Purity: >98%
Handling: Store in Tightly Sealed Vial.
Storage: Room Temperature.
Shipping: Priority Courier
Literature Reference: 1. Merck Index., 1996., 12:, 2085.
Bulk Quantity: Available, Inquire for special pricing

To ADD this item on your order fill in the fields below and select the 'Add to Order' button. Otherwise select the 'Don't Add' button.

Quantity:	Select size:	
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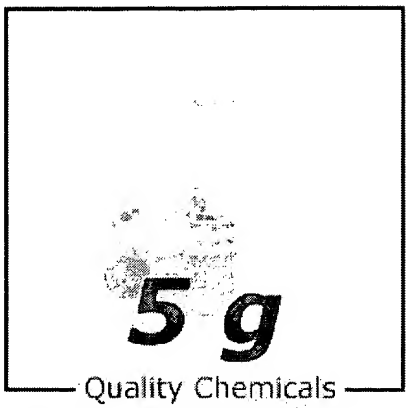

CHAPS, 5G
Brand: BIOPLUS
Model: 0465 @
Catalog #: 730035
Price: $49.90

A zwitterionic nondenaturing detergent suitable for use as a solubilizing agent for membrane proteins because it disrupts non-specific protein interactions. Ref: Hjelmeland, L.H., Proc. Nat. Acad. Sci. USA, 77, 6368 (1980). Ultra Pure Grade. C32H58N2SO7 MW 614.89 CAS# 75621-03-3 Purity (TLC) >98.0% pH (10%, water).

2-Dimensional Electrophoresis - Separation: First Dimension IEF

IPG strip rehydration solution

IPG strips must be rehydrated prior to IEF. The IPG strips are rehydrated in the Immobiline™ DryStrip Reswelling Tray if the Multiphor II™ system is used for IEF, or in IPGphor strip holders, if the IPGphor is used.

Rehydration solution, which may or may not include the sample, is applied to the reservoir slots of the Reswelling Tray or the IPGphor strip holders, then the IPG strips are soaked individually. Rehydrated strips are 3 mm wide and approximately 0.5 mm thick.

Components of the rehydration solution

Selection of the optimal rehydration solution will depend on the specific protein solubility requirements of the sample. A typical solution generally contains urea, non-ionic or zwitterionic detergent, dithiothreitol (DTT), IPG Buffer (Amersham Pharmacia Biotech) appropriate to the pH range of the IPG strip, and dye. The sample may also be included. The role of each component is described below, as well as the recommended concentration range.

- Urea solubilizes and denatures proteins, unfolding them to expose internal ionizable amino acids. Commonly 8 M urea is used, but the concentration can be increased to 9 or 9.8 M if necessary for complete sample solubilization. It has recently been reported that using thiourea in addition to urea further improves solubilization, particularly of membrane proteins.

- Detergent solubilizes hydrophobic proteins and minimizes protein aggregation. The

detergent must have zero net charge—use only non-ionic and zwitterionic detergents. CHAPS, Triton X-100, or NP-40 in a concentration of 0.5 to 4% are most commonly used.

•Reductant cleaves disulphide bonds to allow proteins to unfold completely. DTT or DTE (20 to 100 mM) is commonly used. 2-Mercaptoethanol can be used instead, but higher concentrations are required, and impurities may result in artifacts. It has recently been reported that the non-thiol reductant tributyl phosphine can be used in first-dimension IEF. Add the reductant just prior to use.

•IPG Buffer (carrier ampholyte mixture) can improve separations and sample solubility, particularly with high sample loads. IPG Buffers for each pH range are a mixture of carrier ampholytes that enhances sample solubility and produces more-uniform conductivity across the pH gradient during IEF without affecting the shape of the gradient. IPG Buffers are also specially formulated not to interfere with silver staining. Table 9 lists the recommended final concentration of IPG Buffer for the rehydration solution.

The recommended IPG Buffer concentration for the IPGphor system is 0.5%, but up to 2% can be added if sample solubilization remains a problem.

Note:	Concentrations at the upper end of the recommended range may increase the time required for the voltage to reach its maximum setting during IEF, which can increase the time required for complete focusing.

TABLE 9. ADDITION OF IPG BUFFER TO THE REHYDRATION SOLUTION

IEF system pH range of IPG strip	Suggested carrier ampholytes for rehydration solution	Recommended concentration
Multiphor II 4–7 L, 3–10	IPG Buffer with pH range	2% IPG Buffer (50 µl per 2.5 ml)

L, or 3–10 NL	identical to that of IPG strip	
Multiphor II 6–11 L	pH 6–11 L IPG Buffer	0.5% IPG Buffer (12.5 µl per 2.5 ml)
IPGphor 4–7 L, 3–10 L, 3–10 NL, or 6–11 L	IPG Buffer with pH range identical to that of IPG strip	0.5% IPG Buffer (12.5 µl per 2.5 ml)

IPG Buffer can be included in the stock rehydration solution or added just prior to use. (IPG Buffer is included in the stock solution when multiple IPG strips of the same pH range will be used. IPG Buffer is added just prior to use to single aliquots of the stock solution when the same stock solution will be used with different pH range IPG strips.)

- Tracking dye (Bromophenol Blue) provides a monitor for IEF progress at the beginning of the protocol. If the tracking dye does not migrate toward the anode, no current is flowing. Note, however, that the dye leaves the strip well before the sample is focused!

- Sample can be applied by including it in the rehydration solution. Up to 1 mg of sample per strip can be diluted into or redissolved in rehydration solution just prior to IEF. The amount of sample required is dictated in part by the detection or visualization method used. Radiolabeling requires a very small amount of sample, silver staining requires typically 1 to 100 µg of sample, and Coomassie blue staining and preparative applications require larger amounts.

Rehydration solution preparation

A: Prepare the rehydration stock solution according to one of the following recipes. (Select the formulation appropriate to the experiment.)

Rehydration stock solution without IPG Buffer
(8 M urea, 2% CHAPS, bromophenol blue, 25 ml)

Rehydration st ck solution with ut IPG	Final concentration	Amount

Buffer		
Urea (FW 60.06)	8 M [2]	12 g
CHAPS [3]	2% (w/v)	0.5 g
Bromophenol Blue	trace	(a few grains)
Double distilled H2 O		to 25 ml

Store in 2.5 ml aliquots at -20 °C.

[1] DTT and IPG Buffer are added just prior to use: Add 7 mg DTT per 2.5 ml aliquot of rehydration stock solution. See Table 9 for the appropriate volume of IPG Buffer to use. If loading sample by inclusion in the rehydration solution, sample is also added to the 2.5 ml aliquot of rehydration solution just prior to use.
[2] If necessary, the concentration of urea can be increased to 9 or 9.8 M.
[3] Other detergents (Triton™ X-100, NP-40, and other non-ionic or zwitterionic detergents) can be used instead of CHAPS.

Rehydration stock solution with IPG Buffer
(8 M urea, 2% CHAPS, 0.5% or 2% IPG Buffer [2], Bromophenol Blue, 25 ml)

Rehydration stock solution with IPG Buffer[1]	**Final concentration**	**Amount**
Urea (FW 60.06)	8 M [3]	12 g
CHAPS	2% (w/v)	0.5 g
IPG Buffer (same pH range as the IPG strip)	0.5% or 2% (v/v)	125 or 500 µl
Bromophenol Blue	trace	(a few grains)
Double distilled H_2 O		to 25 ml

Store in 2.5 ml aliquots at -20 °C.

[1] DTT is added just prior to use: 7 mg DTT per 2.5 ml aliquot of rehydration stock solution. If loading sample by inclusion in the

rehydration solution, sample is also added to the 2.5 ml aliquot of rehydration solution just prior to use.
[2] Either of two IPG Buffer concentrations is recommended depending on the IEF system used and the pH range of the IPG strip. Refer to Table 9.
[3] If necessary, the concentration of urea can be increased to 9 or 9.8 M.
[4] Other detergents (Triton X-100, NP-40, and other non-ionic or zwitterionic detergents) can be used instead of CHAPS.
[5] Selection of IPG Buffer concentration is based on IEF system used and pH range of the IPG strip. Refer to Table 9.

[6] Use 125 µl IPG Buffer for a 0.5% concentration and 500 µl IPG Buffer for a 2% concentration.

B Just prior to use, slowly thaw a 2.5 ml aliquot of stock solution. Add the appropriate amount of IPG Buffer, if it is not already included in the rehydration stock solution. (Refer to Table 9).

C Add 7 mg DTT and sample (if desired). Note: DTT and the sample must be added fresh, just prior to use.